QIAGEN Supplementary Protocol:

Isolation of total nucleic acids from animal and human tissues using the EZ1 RNA Tissue Mini Kit

This protocol is designed for the isolation of total nucleic acids (NA) from animal and human tissues using the EZ1 RNA Tissue Mini Kit in combination with the BioRobot® EZ1 workstation.

Introduction

The BioRobot EZ1 RNA system allows fully automated purification of RNA from animal and human tissues. In brief, total nucleic acids are isolated from sample lysates, then DNA is removed by DNase digestion; this is followed by washing and elution of pure RNA. **In this supplementary protocol, however, the DNase treatment step is omitted, to allow purification of high-quality total nucleic acids (DNA and RNA) in high yields.**

The high-quality nucleic acids obtained using BioRobot EZ1 are well suited for direct use in downstream applications, such as amplification or other enzymatic reactions. The BioRobot EZ1 performs all steps of the purification procedure.

The procedure given below describes the preparation of lysate from animal or human soft tissue, and the subsequent procedure for setting up the BioRobot EZ1 and starting a run.

IMPORTANT: Please read the *EZ1 RNA Handbook*, paying careful attention to the "Safety Information" and "Important Notes" sections, before beginning this procedure.

Starting material

The EZ1 RNA Tissue Mini Kit is optimized for purification of nucleic acids from 1 to 10 mg soft tissue. If you use more than the maximum recommended amount of starting material, you may not achieve further increases in nucleic acid yields. The starting and elution volumes to use in this procedure are given in Table 1.

Table 1. Amount of Starting Material and Elution Volumes Used with the EZ1 RNA Tissue Mini Kit and the Total Nucleic Acid Protocol

Sample	EZ1 RNA Card Protocol	Amount of starting material	Elution volume
Soft tissue*	Total Nucleic Acid	1–10 mg†	500–200 µl

* For example, liver and kidney.

† Sample volume: 300 µl.

Yield of purified nucleic acids

Yields may vary depending on the elution volume used. Elution in smaller volumes increases the final concentration of nucleic acids in the eluate, but slightly reduces the overall yield. We recommend using an elution volume appropriate for the intended downstream application. Typical yields of total nucleic acids are shown in Table 2, below.

Table 2. Yields of Nucleic Acids Obtained from Tissue Using the EZ1 RNA Tissue Mini Kit and the Total Nucleic Acid Protocol

Sample type	Amount of tissue	Yield (µg NA)
Pig liver*	1 mg	7.5
Pig liver*	5 mg	30

* Total nucleic acids were eluted in 200 µl RNase-free water.

Equipment and reagents to be supplied by user

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

- EZ1 RNA Card, cat. no. 9015590
- EZ1 RNA Tissue Mini Kit, cat. no. 959034
- BioRobot EZ1 workstation, cat. no. 9000705, and disposables (see the *EZ1 RNA Handbook*)
- 14.3 M β-mercaptoethanol (β-ME)† (commercially available solutions are usually 14.3 M)
- Sterile, RNase-free pipet tips
- Disposable gloves
- Equipment for disruption and homogenization (see "Disrupting and homogenizing starting material" in the *EZ1 RNA Handbook*)

For suppliers of bead-mill homogenizers and rotor–stator homogenizers, see "Equipment and Reagents to Be Supplied by User" in the *EZ1 RNA Handbook)*.

† β-ME is added to Buffer RLT before use (see "Things to do before starting", below).

Important points before starting

- If using the EZ1 RNA Tissue Mini Kit for the first time, read "Important Notes" in the kit handbook.
- If working with RNA for the first time, read Appendix A in the kit handbook.
- For best results, stabilize animal tissues immediately in RNA*later*® RNA Stabilization Reagent. Tissues can be stored in RNA*later* TissueProtect Tubes for up to 1 day at 37°C, 7 days at 18–25°C, 4 weeks at 2–8°C, or for archival storage at –20°C or –80°C. See the *RNA*later *Handbook* for more information about RNA*later* RNA Stabilization Reagent and about stabilizing RNA in tissues.
- Fresh, frozen, or RNA*later* stabilized tissue can be used. To freeze tissue for long-term storage, flash-freeze in liquid nitrogen, and immediately transfer to –70°C. Tissue can be stored for several months at –70°C. To process, do not allow tissue to thaw during weighing or handling before disruption in Buffer RLT. Homogenized tissue lysates (in Buffer RLT, step 4) can also be stored at –70°C for several months. To process frozen lysates, thaw samples at room temperature or at 37°C in a water bath until they are completely thawed and salts in the lysis buffer are dissolved. Avoid extended treatment at 37°C, which can cause chemical degradation of the RNA. Continue with step 5.
- Buffer RLT and reagent cartridges contain a guanidine salt and are therefore not compatible with disinfecting reagents containing bleach. See "Safety Information" in the kit handbook. Take appropriate safety measures and wear gloves when handling.
- All steps of the protocol should be performed at room temperature (15–25°C). During the procedure, work quickly.
- RNase-free DNase and Buffer RDD are not required in this procedure.

Things to do before starting

- Buffer RLT may form a precipitate upon storage. If necessary, redissolve by warming and then place at room temperature.
- Add 10 µl β-ME per 1 ml Buffer RLT. Dispense in a fume hood and wear appropriate protective clothing. Buffer RLT is stable at room temperature for 1 month after addition of β-ME.

Procedure

1. **Excise the tissue sample from the animal or remove it from storage. Remove RNA*later* stabilized tissues from the reagent using forceps.**
2. **Determine the amount of tissue. Do not use more than 10 mg. Proceed immediately with step 3.**

   Weighing tissue is the most accurate way to determine the amount.

   Note: For tissues of high cell density, such as spleen, do not use more than 5 mg.

3. **For RNA*later* stabilized tissues:**

   If the entire piece of RNA*later* stabilized tissue can be used for isolation of total nucleic acids, place it directly into a suitably sized vessel for disruption and homogenization, and proceed with step 4.

   If only a portion of the RNA*later* stabilized tissue is to be used, place the tissue on a clean surface for cutting, and cut it. Determine the weight of the piece to be used, and place it into a suitably sized vessel for homogenization. Proceed with step 4.

   RNA in the RNA*later* treated tissue is still protected while the tissue is processed at 18–25°C. This allows cutting and weighing of tissues at ambient temperatures. It is not necessary to cut the tissue on ice or dry ice or in a refrigerated room. The remaining tissue can be placed into RNA*later* RNA Stabilization Reagent for further storage. Previously stabilized tissues can be stored at –80°C without the reagent.

   For unstabilized fresh or frozen tissues:

   If the entire piece of tissue can be used for isolation of total nucleic acids, place it directly into a suitably sized vessel for disruption and homogenization, and proceed immediately with step 4.

   If only a portion of the tissue is to be used, determine the weight of the piece to be used, and place it into a suitably sized vessel for homogenization. Proceed immediately with step 4.

   RNA in tissues is not protected after harvesting until the sample is treated with RNA*later* RNA Stabilization Reagent, flash-frozen, or disrupted and homogenized in protocol step 4. Frozen animal tissue should not be allowed to thaw during handling. The relevant procedures should be carried out as quickly as possible.

   Note: The remaining fresh tissue can be placed into RNA*later* RNA Stabilization Reagent for stabilization (see *RNA*later *Handbook*). However, previously frozen tissue samples thaw too slowly in the reagent, thus preventing it from diffusing into the tissue quickly enough before the RNA begins to degrade.

4. Disrupt tissue and homogenize lysate in Buffer RLT (do not use more than 10 mg tissue). Disruption and homogenization of tissue can be performed by 3 alternative methods (4a, 4b, or 4c).

See "Disruption and homogenization of starting material" in the *EZ1 RNA Mini Handbook* for a more detailed description of disruption and homogenization methods.

After storage in RNA*later* RNA Stabilization Reagent, tissues may become slightly harder than fresh or thawed tissues. Disruption and homogenization of tissue samples using standard methods is usually not a problem.

Note: Incomplete homogenization will lead to significantly reduced yields. Homogenization with rotor–stator homogenizers generally results in higher total nucleic acid yields than with other homogenization methods.

4a. Rotor–stator homogenization:

Place the weighed (fresh, frozen, or RNA*later* stabilized) tissue in a suitably sized vessel for the homogenizer. Add 300 µl Buffer RLT. Homogenize immediately using a conventional rotor–stator homogenizer until the sample is uniformly homogeneous (usually 20–40 s). Continue the protocol with step 5.

Rotor–stator homogenization simultaneously disrupts and homogenizes the sample.

4b. Mortar and pestle with QIAshredder homogenization:

Immediately place the weighed (fresh, frozen, or RNA*later* stabilized) tissue in liquid nitrogen, and grind thoroughly with a mortar and pestle. Decant tissue powder and liquid nitrogen into an RNase-free, liquid-nitrogen–cooled, 2 ml microcentrifuge tube (not supplied). Allow the liquid nitrogen to evaporate, but do not allow the tissue to thaw.

Add 300 µl Buffer RLT. Pipet the lysate directly onto a QIAshredder spin column placed in a 2 ml collection tube, and centrifuge for 2 min at maximum speed. Continue the protocol with step 5.

Grinding the sample using a mortar and pestle will disrupt the sample, but it will not homogenize it. Homogenization is carried out by centrifugation through the QIAshredder Spin Column.

4c. TissueLyser homogenization:

Place the weighed (fresh, frozen, or RNA*later* stabilized) tissue in a 2 ml microcentrifuge tube (not supplied), add 300 µl Buffer RLT, and add one stainless steel bead (3–7 mm diameter). Homogenize on the TissueLyser for 2 min at 20 Hz. Rotate the TissueLyser rack, and homogenize for another 2 min at 20 Hz. Continue the protocol with step 5.

Note: The instructions in step 4c are only guidelines. They may need to be changed depending on the sample being processed and on the bead mill being used.

5. Centrifuge the tissue lysate for 3 min at maximum speed in a microcentrifuge. Carefully transfer the supernatant to the 2 ml sample tubes supplied with the kit.

6. Insert the EZ1 RNA Card completely into the EZ1 Card slot of the BioRobot EZ1.

7. Switch on the BioRobot EZ1.

8. Press "START" to display the "Protocols" menu.

9. Press "2" to select purification of total nucleic acids.

10. Press "2" to select purification of total nucleic acids from tissue.

11. **Press any key to proceed through the text displayed in the LCD.**
12. **Press "1" to select an elution volume of 50 µl, press "2" to select an elution volume of 100 µl, or press "3" to select an elution volume of 200 µl.**
13. **Press any key to proceed through the text displayed in the LCD.**

    The text summarizes the following steps that describe the loading of the worktable.
14. **Open the workstation door.**
15. **Invert 1–6 reagent cartridges twice to mix the magnetic particles. Then tap the cartridges to deposit the reagents at the bottom of their wells.**
16. **Load the reagent cartridges into the cartridge rack.*†**

    Note: After sliding a reagent cartridge into the cartridge rack, ensure that you press down on the cartridge until it clicks into place.

    If there are fewer than 6 reagent cartridges, you can load them in any order on the rack. However, when loading the other labware in steps 17–19, ensure that they also follow the same order.
17. **Load 1–6 opened elution tubes into the elution tube rack.†**
18. **Load 1–6 tip holders containing filter-tips into the front 2 rows of the tip rack.†**
19. **Load 1–6 opened sample tubes containing lysate (from step 4) into the back row of the tip rack.†**
20. **Close the workstation door.**
21. **Press "START" to start the protocol.**
22. **When the protocol ends, the LCD displays "Protocol finished". Open the workstation door.**
23. **Remove the elution tubes containing the purified total nucleic acids. Discard the sample-preparation waste.**
24. **To run another protocol, press "ESC", prepare samples as described in steps 1–5, and follow the procedure from step 9 onward. Otherwise, press "STOP" twice to return to the first screen of the LCD, close the workstation door, and switch off the BioRobot EZ1.**
25. **Clean the BioRobot EZ1.**

    Follow the maintenance instructions in the *BioRobot EZ1 User Manual*.

Troubleshooting

For troubleshooting, please consult the Troubleshooting Guide in the kit handbook.

* See Figure 2B in the kit handbook.

† See Figure 3 in the kit handbook.

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